Carbohydrate Research, 81 (1980) 177–181

Note

N.m.r. investigation of specifically labeled [^{13}C]nigeran

THOMAS F. BOBBITT, JOHN H. NORDIN,
Department of Biochemistry, University of Massachusetts, Amherst, MA 01003 (U.S.A.)

DIDIER GAGNAIRE,
Centre de Recherches sur les Macromolécules Végétales (C.N.R.S.), 53 X, 38041 Grenoble Cédex (France)

AND MARC VINCENDON
Laboratoire de Chimie Organique Physique, Département de Recherche Fondamentale, Commissariat à l'Energie Atomique, 85 X, 38041 Grenoble Cédex (France)

(Received March 30th, 1979; accepted for publication in revised form, August 15th, 1979)

Nigeran is a regular copolysaccharide having alternating (1→3)- and (1→4)-α-D-glucopyranosyl residues. The repeating unit is therefore a disaccharide. Chemical[1], X-ray diffraction, and chain-conformation analyses[2,3] have shown that nigeran is a regular, linear glucan.

The ^{13}C-n.m.r. spectrum of nigeran may therefore be expected to give 12 signals, corresponding to the basic repeating-unit. Fig. 1 shows the ^{13}C spectrum obtained at 62.86 MHz in dimethyl sulfoxide-d_6 solution at 80° (because of high viscosity of the solution, a poorly resolved spectrum is obtained at room temperature). It shows 11 signals, one of which (δ 71.4 p.p.m.) is twice as intense as the others and corresponds to two carbon atoms. The main feature of this spectrum is that each pair of signals, the anomeric signals at 100 p.p.m., signals of the glycosidic carbon atoms at 80 p.p.m., and the C-6 hydroxymethyl signals at 60 p.p.m., shows an intensity difference. This difference is independent of *temperature*, *molecular weight* (so long as this remains >20,000), and *magnetic field*. This is the first time that such a phenomenon has been described for a linear copolysaccharide*. Neither lichenan [(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*] nor pullulan[5] [(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→6)-α-D-Glc*p*] show such a feature in their ^{13}C spectra obtained under the same conditions[6].

The difference in intensities of the signals of two carbon atoms linked to the same number of protons and having the same environment may come from (*a*) a T_1 relaxation-time difference and, because of a main dipole–dipole relaxation phe-

---

*Seymour *et al.* have recently noticed a similar phenomenon with branched dextrans; see ref. 4.

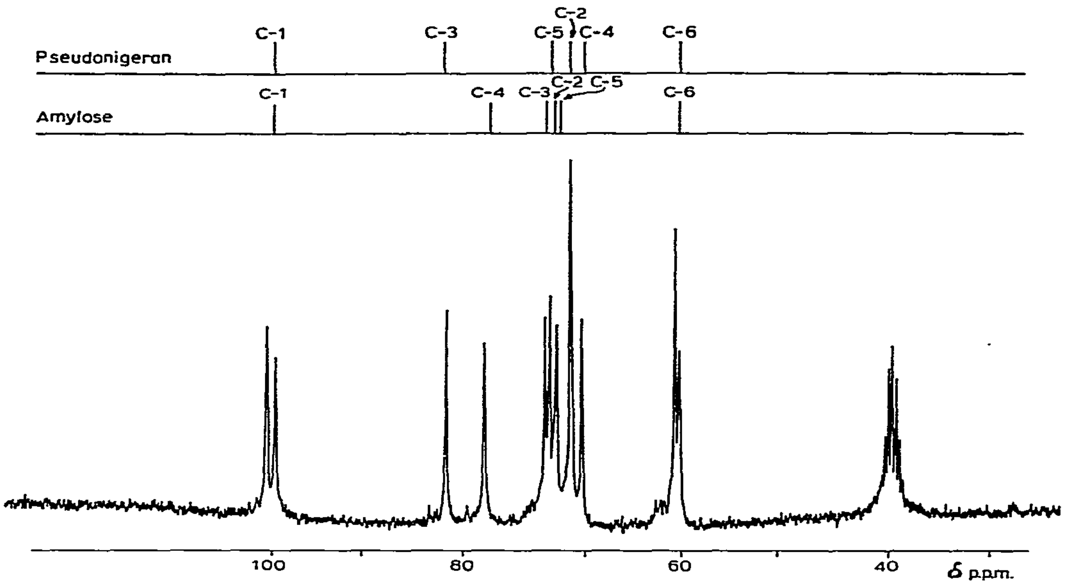

Fig. 1. The 62.86-MHz ^{13}C spectrum of nigeran (8%) in dimethyl sulfoxide-d_6 solution; T, 80°; sweep width, 12,500 Hz; acquisition time, 0.65 sec; pulse width, 10 μsec; 16K memory: number of scans, 80,000.

nomenon, from a correlation time of segmental-motion difference; or (*b*) differences in nuclear Overhauser enhancement (n.O.e.).

Another important question is: do the more-intense signals arise from one specific glucose residue or from both? To answer this question, a full assignment of the spectrum was necessary.

Tentative assignment. — The ^{1}H spectrum of nigeran is rather complicated. All proton signals except those of anomeric protons are located between 3 and 4 p.p.m. It is therefore very difficult to assign the proton spectrum. Heteronuclear spin-decoupling thus cannot be used to assign the ^{13}C spectrum.

However, the deuterium hydroxyl isotopic effect[6,7] allows the differentiation of carbon atoms bearing hydroxyl groups ($\Delta\nu = 15\text{–}20$ Hz) from those not having this substituent, generally C-5 ($\Delta\nu = 10$ Hz).

A comparison of the ^{13}C signals of nigeran with those of the corresponding homopolymers (Fig. 1) amylose [(1→4)-α-D-Glc*p*] and pseudonigeran [(1→3)-α-D-Glc*p*] gives good indication of all signals, except for those arising from the anomeric and C-6 carbon atoms.

^{13}C-Labeled nigeran. — ^{13}C-Enriched nigeran was prepared from D-[1-^{13}C]-glucose labeled to the extent of 86% at C-1 (CEA Saclay, France) by growing *Aspergillus aculeatus* on this carbon source[8]. From 1.5 g of D-glucose, 0.3 g of polymer was obtained. Fig. 2 gives the ^{13}C spectrum of ^{13}C-enriched nigeran. This spectrum shows that the two C-1 atoms are the more heavily labeled, but are not the only ones labeled. The C-6 atoms are also labeled to a certain extent, whereas the remaining carbon atoms have only the natural ^{13}C abundance.

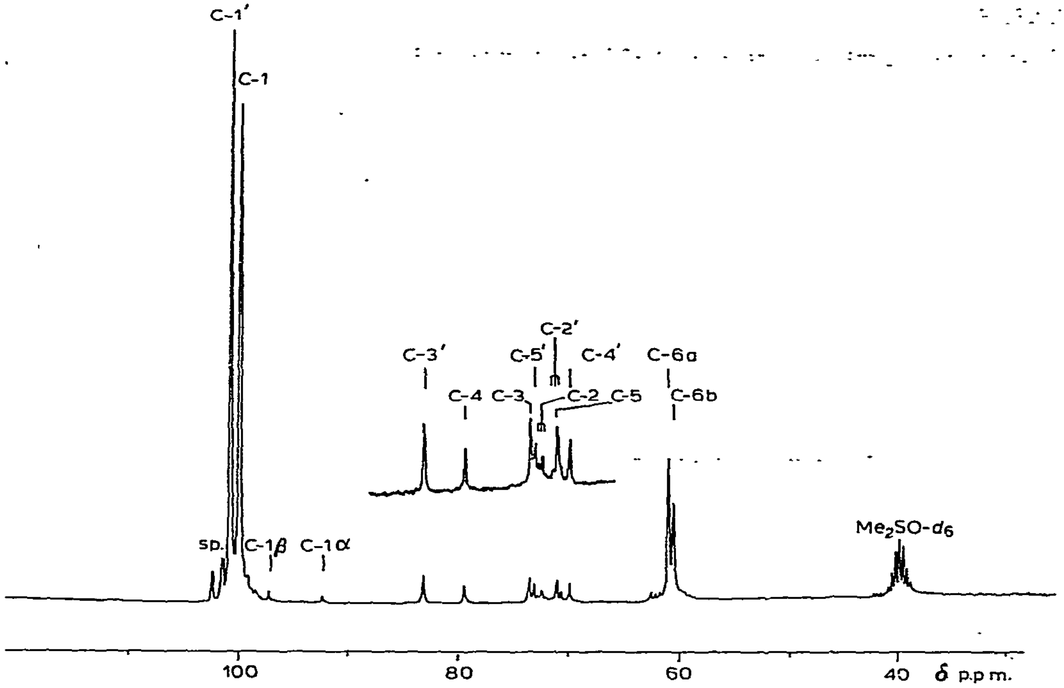

Fig. 2. The ^{13}C spectrum of enriched nigeran: same conditions as in Fig. 1. The primed numbers refer to the (1→3)-α-D-glucopyranosyl residue.

The proton-n.m.r. spectrum of enriched nigeran permits calculation of the proportion of ^{13}C at C-1 through examination of ^{13}C satellites. The integrated ratio of ^{13}C–H-1 proton signals (^{13}C–H-1 + ^{12}C–H-1) gives the amount of ^{13}C remaining on the two C-1 positions of enriched nigeran. The average of six integrals gives 55.6 and 56% for the two positions. This result means that the two C-1 positions are equally labeled and that a common D-glucose pool serves for formation of the precursor(s) of the (1→3)- and (1→4)-linked D-glucosyl residues.

The absolute value of 56% for ^{13}C at C-1 was used as a reference to determine the relative amount of ^{13}C at the other positions. This determination was made by using the ^{13}C-n.m.r. spectrum with a long delay between two pulses and by suppressing the n.O.e. by decoupling during the acquisition time. A correction was made to take into account differences of signal intensities with natural nigeran under the same experimental conditions.

Abundance values of 9.2 and 16.5% of ^{13}C were found at C-6a and C-6b, showing that these positions have respectively 8 and 10 times the natural ^{13}C abundance. This result indicates that a certain proportion of the D-glucose molecules involved as precursors of nigeran are resynthesized from triose phosphates derived directly *via* glycolysis from D-[1-^{13}C]glucose and equilibrated *via* triose phosphate isomerase.

Signals that appear in the range of 70–74 p.p.m. are slightly modified through

TABLE I

^{13}C-CHEMICAL SHIFT[a] OF NIGERAN IN Me_2SO-d_6 SOLUTION AT 80°

Linkage	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
α-(1→4)	100	72.7	73.9	79.8	71.4	61.3 60.9
α-(1→3)	101	71.4	83.2	70.3	73.5	60.9 61.3

[a] Relative to internal tetramethylsilane.

TABLE II

^{13}C–H AND ^{13}C–^{13}C COUPLING CONSTANTS OF NIGERAN

J (Hz)	*α-(1→3)*	*α-(1→4)*
$J_{H\text{-}1,H\text{-}2}$[a]	3.25	3
$J_{C\text{-}1,H\text{-}1}$[a]	169	169
$J_{C\text{-}1,H\text{-}1}$	169.5 (171)	168 (169)
$J_{C\text{-}3,H\text{-}3}$	147.5 (149.5)	145 (141)
$J_{C\text{-}4,H\text{-}4}$	152.5 (158.5)	141 (140)
$J_{C\text{-}6,H\text{-}6}$	(C-6a) 140.5	(C-6b) 141 (139)
$J_{C\text{-}1,C\text{-}2}$	43	47

[a] Determined from the proton spectrum. Values in parentheses are relative to the homopolymers.

^{13}C–^{13}C coupling. These modifications allow the identification of the two C-2 signals at 72.7 and 71.4 p.p.m., the more deshielded being assigned to the (1→4)-α-Glc*p* residue by comparison with the homopolymer (amylose).

Experiments on ^{13}C–^{13}C decoupling with labeled nigeran permitted tentative assignment of the anomeric carbon atoms through $J_{C\text{-}1,C\text{-}2}$ coupling constants.

Thus all of the carbon signals were assigned (Table I), except for the C-6 atoms, for which the coupling with 10% of C-5 is not sufficient to produce a modification of the spectrum in ^{13}C–^{13}C, homonuclear spin-decoupling experiments.

By using the intensities of the free anomeric signals of the chain ($\delta_{C\text{-}1\beta} = 97.3$ p.p.m. and $\delta_{C\text{-}1\alpha} = 91.9$ p.p.m.), an average $\overline{\text{d.p.}}_n$ value of 90 was found.

Table II summarizes the $J_{^{13}C,H}$ and $J_{^{13}C,^{13}C}$ coupling constants obtained from the proton, ^{13}C-coupled and ^{13}C-decoupled spectra of labeled nigeran.

In conclusion, nigeran has been selected as a typical example of a regular, alternating, linear copolysaccharide giving differences of ^{13}C signal-intensities. The possibility of obtaining [^{13}C]nigeran selectively labeled at C-1 and C-6 of both residues will allow more precise T_1, T_2, and n.O.e. determinations, as will variation of parameters (magnetic field, concentration, and temperature), as has been shown important for other polymers[9].

ACKNOWLEDGMENTS

We are grateful to R. Nardin and H. Reuternauer from the Laboratoire Grenoblois de Résonance Magnétique for technical assistance. We thank the Université Scientifique et Médicale de Grenoble for financial support to two of us (D.G. and M.V.).

REFERENCES

1 S. A. Barker, E. J. Bourne, and M. Stacey, *J. Chem. Soc.*, (1953) 3084–3090.
2 D. A. Rees, *J. Chem. Soc.*, *B*, (1969) 217–221.
3 P. R. Sundararajan, R. H. Marchessault, G. J. Quigley, and A. Sarko, *J. Am. Chem. Soc.*, 95 (1973) 2001–2008.
4 F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 72 (1979) 229–234.
5 P. Colson, H. J. Jennings, and I. C. P. Smith, *J. Am. Chem. Soc.*, 96 (1974) 8081–8087.
6 D. Gagnaire and M. Vincendon, *Bull. Soc. Chim. Fr.*, (1977) 479–482.
7 D. Gagnaire and M. Vincendon, *J. Chem. Soc., Chem. Commun.*, (1977) 509–510.
8 D. Gagnaire, D. Mancier, and M. Vincendon, *Org. Magn. Resonance*, 11 (1978) 344–349.
9 T. F. Bobbitt, J. H. Nordin, M. Roux, J. F. Revol, and R. H. Marchessault, *J. Bacteriol.*, 132 (1977) 691–703.
10 G. C. Levy, D. E. Axelson, R. Schwartz, and J. I. Hochman, *J. Am. Chem. Soc.*, 100 (1978) 410–424.